Journal of Great Lakes Research xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

A novel method for tracking western Lake Erie *Microcystis* blooms, 2002–2011

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ARTICLE INFO

Article history:
Received 26 April 2012
Accepted 23 November 2012
Available online xxxx

Communicated by Joseph Makarewicz

Keywords:
Harmful algal blooms
Eutrophication
Lake Erie
Microcystis
Cyanobacteria

ABSTRACT

After a period of improvement from the late 1970s through the mid 1990s, western Lake Erie has returned to eutrophic conditions and harmful algal blooms now dominated by the cyanobacterium *Microcystis aeruginosa*. The detection of long-term trends in *Microcystis* blooms would benefit from a convenient method for quantifying *Microcystis* using archived plankton tows. From 2002 to 2011, summer *Microcystis* blooms in western Lake Erie were quantified using plankton tows (N = 649). A flotation separation method was devised to quantify *Microcystis* biovolume in the tows, and the method was tested against whole water cell counts. Floating *Microcystis* biovolume (mL) in preserved tows was highly correlated with total *Microcystis* cells ($R^2 = 0.84$) and biomass ($R^2 = 0.95$) in whole water samples. We found that *Microcystis* annual biovolume was highly variable among years; the 2011 bloom was 2.4 times greater than the second largest bloom (2008) and 29.0 times greater than the smallest bloom (2002). Advantages of the method include use of archived samples, high sampling volume, and low effort and expense. Limitations include specificity for cyanobacterial blooms dominated by large *Microcystis* colonies and the need for site-specific validation. This study indicates that the flotation method can be used to rapidly assess past and present *Microcystis* in western Lake Erie and that there was high variability in the timing, duration, and intensity of the annual *Microcystis* blooms over a 10-year period. The data made possible by this method will aid further investigations into the underlying causal factors of blooms.

Introduction

The history of cultural eutrophication in Lake Erie is well known and has been the subject of extensive research over the past 50 years. The mid-1900s were decades of declining ecosystem health that culminated in massive algal blooms in the western basin of the lake and increasing hypoxia in the central basin (Burns, 1985; Vallentyne, 1974; among others). Following the Great Lakes Water Quality Agreement (GLWQA) in 1972 that set maximum target phosphorus (P) loads, conditions began to improve in the late 1970s and early 1980s. By 1987, biomass of phytoplankton, especially the dominant cyanobacterium *Aphanizomenon flos-aquae*, was considerably reduced (Makarewicz, 1993) and by the early 1990s, large blooms of cyanobacteria had become rare in Lake Erie (Makarewicz et al., 1999). In the mid-1990s, however, western Lake Erie entered a period of re-eutrophication as phytoplankton biomass increased (Conroy and Culver, 2005) and summer blooms, now dominated by the cyanobacterium *Microcystis*, began to reappear (Brittain et al., 2000; Budd et al., 2002). *Microcystis aeruginosa* accounts for 95% of the blooms in western Lake Erie, but other species of *Microcystis* may be present (Millie et al., 2009). Hereafter, we refer to *Microcystis* spp. collectively as "*Microcystis*." *Microcystis* blooms are of concern largely because of their production of hepatotoxic microcystins. Concentrations of microcystins in western Lake Erie during summer bloom periods have been found to exceed the 1-μg/L safety limit for drinking water established by the World Health Organization (Rinta-Kanto et al., 2005; Wilson et al., 2008), and municipalities that depend on the lake as a primary source of drinking water may spend over $100,000 per month in additional treatment costs to ensure the safety of the water supply (Raymond, 2012).

Long-term data sets that quantify *Microcystis* blooms and environmental factors such as nutrient loading are needed in order to understand the relationships between blooms and the environment. Unfortunately, quantifying *Microcystis* blooms can be challenging. There are five common methods of quantifying *Microcystis*: microcystin concentration, chlorophyll *a* concentration, cell counts, quantitative real-time PCR (qPCR), and remote sensing. Each method has advantages and disadvantages. *Microcystis* monitoring efforts frequently focus on the concentration of the toxin found in lake water (Boyer, 2008). However, toxin concentration may vary with the percentage of toxic vs. non-toxic *Microcystis* strains present and is often not well correlated with cell counts (Dyble et al., 2008); therefore it is not always a reliable surrogate for *Microcystis* abundance. Chlorophyll *a* concentration is frequently used as a surrogate for total phytoplankton biomass, but it cannot differentiate among different taxonomic groups. Cell counts are used as direct measurement of abundance, but they are time-consuming, which limits the number of measurements that can be obtained. Furthermore, *Microcystis* is frequently found in colonies of >10,000 cells, which necessitates

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0380-1330/$ – see front matter
http://dx.doi.org/10.1016/j.jglr.2012.11.004

Please cite this article as: Bridgeman, T.B., et al., A novel method for tracking western Lake Erie *Microcystis* blooms, 2002–2011, J Great Lakes Res (2012), http://dx.doi.org/10.1016/j.jglr.2012.11.004

estimates of cell numbers from measurements of colony dimensions. Increasingly, qPCR is becoming useful in quantifying *Microcystis*, particularly in distinguishing between toxic and non-toxic genotypes (Rinta-Kanto et al., 2005). However this technique requires a substantial investment in analytical equipment and training, and conversion of qPCR results to cell counts is problematic (Rinta-Kanto et al., 2005). Remote sensing, particularly via the satellite-based MODIS, MERIS, and LANDSAT sensors, is useful in delineating broad areas of surface *Microcystis* blooms over time (Wynne et al., 2008) or its associated pigments (Becker et al., 2009; Vincent et al., 2004). However, the results from satellite images are highly dependent on the concentration of gas vacuoles within the cells of cyanobacteria and the vertical distribution of colonies in the water column (Ganf et al., 1989), and may also vary with the presence of other algal species that can contain the same pigments (Hill and Rowan, 1989).

In this study we developed a novel method that uses formalin-preserved vertical plankton tows to quantify *Microcystis* in the water column. Our flotation separation method takes advantage of *Microcystis'* buoyancy when preserved in sugar formalin and allows the analysis of archived plankton tows from previous years. After establishing the effectiveness of the method, we used it to analyze archived plankton tows collected by our laboratory in a long-term western Lake Erie water quality monitoring program dating to 2002. The large number of plankton tows analyzed in this manner between 2002 and 2011 allowed us to make spatial, seasonal, and inter-annual comparisons that, in combination with environmental data over the same period, will be useful in explaining the major causes of *Microcystis* blooms in this system. Here, we 1) present a method to quantify *Microcystis* in plankton net samples, 2) test the efficiency of the method by determining how well *Microcystis* separates from other plankton, 3) compare the method to more traditional quantification methods, and 4) compare the *Microcystis* blooms of western Lake Erie through space and time.

Methods

Site description

This study was conducted in western Lake Erie in the vicinity of Maumee Bay. The area is strongly influenced by the inflow of the Maumee River, which contributes high loadings of suspended sediments (Richards et al., 2008) and nutrients (Baker and Richards, 2002) to the bay and lake. Six sampling locations in Maumee Bay and western Lake Erie (Fig. 1) were chosen to sample along the gradient of water quality produced by the outflow of the Maumee River. The two bay sites, MB20 (N 41°71.498, W83°45.59) and MB18 (N 41°74.207, W 83°40.16), have depths of less than 2.5 m and are highly influenced by the outflow of the Maumee River. Turbidity and nutrient concentrations are very high in the bay (Secchi depths < 30 cm and total phosphorus > 0.150 mg/L) and decline with distance into the lake (Moorhead et al., 2008). Two intermediate sites, 8M (N 41°78.901, W 83°35.56) and 7M (N 41°73.340, W 83°29.71), have depths of 5–6 m with characteristics that indicate Maumee River influence. Two off-shore sites, GR1 (N 41°82.078, W 83°18.56) and 4P (N 41°75.045, W 83°10.35), have depths of 8.5 m and 10 m respectively and are the least affected by the Maumee River. Site GR1 usually has relatively clear water with low nutrient concentrations, indicating influence from the upper Great Lakes via the Detroit River.

Microcystis biovolume

Every 10 to 20 days, beginning in mid May, *Microcystis* and other plankton were collected at each site between the hours of 9 am and 4 pm in vertical tows from near the lake bottom to the surface using a 0.5-m diameter, 112-μm mesh net equipped with a flow meter to calculate net efficiency. All plankton tows were concentrated to a volume of 100 mL to 500 mL and immediately preserved in 4% buffered (pH = 7.5) sugar-formalin. All tows were collected in duplicate and stored in the laboratory at room temperature until analysis. In all, a total of 649 plankton tows collected between May and October (2002–2011) were analyzed.

Microcystis biovolume from preserved plankton tows was determined by allowing the positively buoyant *Microcystis* colonies to separate from the remaining plankton in graduated 1 L Imhoff cones. Entire plankton samples were poured into Imhoff cones and diluted with tap water to 200–1000 mL, depending on the amount of *Microcystis* and other plankton in the samples. After 24 h, settled zooplankton and non-buoyant phytoplankton were drawn out of the bottom of the cone and preserved, leaving a concentrated layer of floating *Microcystis* in the upper graduated portion of the cone. After a second 24-hour settling period, the volume of *Microcystis* was recorded to the nearest 0.1 mL, when less than 10 mL of *Microcystis* was present, or to nearest

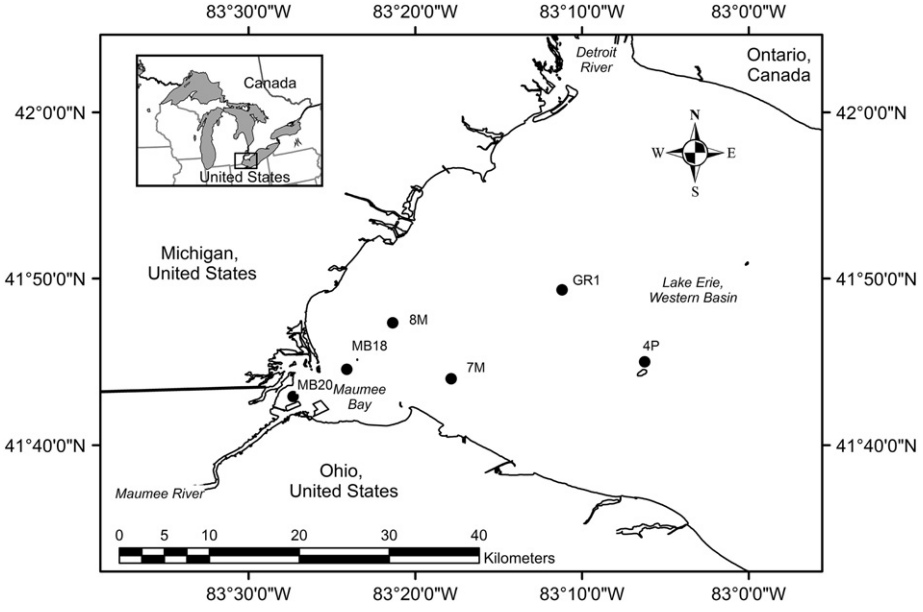

Fig. 1. Sampling locations in western Lake Erie during 2002–2011.

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1.0 mL when more than 10 mL of *Microcystis* was present. In a subset of trials (N = 5), no further change in *Microcystis* volume was observed after allowing samples to settle for an additional day; therefore 24 h was judged to be an adequate settling period. Because *Microcystis* concentration may vary over lake depth, we calculated *Microcystis* biovolume on an areal basis. To obtain an areal estimate of *Microcystis* (mL/m^2) present at each sample location and date, the average biovolume (mL) from the replicate tows measured in Imhoff cones was divided by the area of the plankton net (0.196 m^2).

Microcystis separation efficiency

The efficiency of *Microcystis* separation using the flotation method depends upon *Microcystis* colonies being positively buoyant and also not becoming entangled with other plankton. To determine the efficiency of the flotation method, colony counts were performed before and after separation on a subset of sugar formalin-preserved plankton samples (N = 60). Samples were selected across a range of sites, months, and years in storage ranging from 1 month to 5 years. Each sample was diluted to 1000 mL, mixed thoroughly, and the initial density of *Microcystis* colonies was determined by counting colonies in a 1-mL subsample using a Bogorov counting chamber under a dissecting microscope (Leica MZ12, 25× magnification). After the first 24-hour settling period, the non-buoyant phytoplankton and zooplankton fraction was drawn out through the bottom of the Imhoff cone into a beaker. This negatively buoyant fraction contained *Microcystis* colonies that failed to separate by flotation because they had ruptured or unfilled gas vacuoles or became entangled with filamentous phytoplankton. The density of *Microcystis* colonies in this fraction was determined after diluting the fraction to 1000 mL and counting the number present in a 1-mL subsample as before. The percent separation achieved was calculated as 1 − (number of colonies in negatively buoyant fraction / number of colonies in the whole sample) × 100. To increase separation efficiency and to obtain a more accurate estimate of *Microcystis* in samples with a high amount of filamentous plankton, a second separation was performed on the non-buoyant fraction of all plankton samples used in this study. The non-buoyant portion was added to another Imhoff cone and diluted, which allowed the *Microcystis* colonies to untangle from other plankton and float. *Microcystis* bioviolume of the two separations were summed. The separation efficiency was only determined for the initial separation; therefore the total separation efficiency after a second separation would be somewhat greater than what we report.

Effectiveness of capturing Microcystis with a plankton net

To determine whether net plankton samples can be used to accurately quantify colonial *Microcystis* in western Lake Erie, we compared our method to the standard method of performing cell counts on whole water samples (not strained through a mesh). Whole water samples have a potential advantage in that small colonies are not lost during collection. During 2011, whole water column samples (surface to near sediments) were collected using integrated tube-samplers in parallel with the plankton net tows (N = 15). Lake water from the tube-sampler was poured into 400-mL glass jars and then fixed in a 1% Lugol's solution. In the laboratory, the preserved samples were settled in graduated cylinders for 48 h to concentrate them to 30 mL. To determine *Microcystis* cell density, the concentrated 30-mL sample was mixed and a 1-mL subsample was pipetted into a gridded Sedgewick Rafter slide (Wetzel and Likens, 2000). *Microcystis* cell density (cells/mL) was quantified by measuring the area of every colony in the 1-mL sample using a digital camera and software (Davis et al., 2012) and applying the relationship between colony area and number of cells developed by Watzin et al. (2006). Regression analysis between biovolume and cell density was performed using SAS.

Retention of Microcystis by net tows with different mesh sizes

A plankton net with mesh size of 112 μm was used throughout our study. To compare the ability of this mesh size to retain *Microcystis* colonies to a finer mesh size, during 2008 we collected paired plankton samples (N = 13 pairs) using both the 112-μm mesh net and a 64-μm mesh net. *Microcystis* biovolume collected in the two nets was determined as above.

Comparing the flotation technique to other methods

In many studies, phytoplankton is quantified by cell density or mass. To make our biovolume data useful for comparison, we determined the number of cells (for N = 60 samples) and dry mass (for N = 20 samples) of the floating *Microcystis* portion. Samples were randomly selected from all years, months when *Microcystis* was present, and sites and ranged from low to high *Microcystis* biovolume. To perform cell counts, the floating *Microcystis* portion was drawn into a beaker after separation, diluted to 1000 mL, and boiled for 6 min to break apart colonies into individual cells for ease of counting. Boiling has been shown to be effective in breaking up *Microcystis* colonies without producing cell degradation (Joung et al., 2006). After cooling and mixing the sample thoroughly, a 0.1-mL subsample was added to a Palmer counting cell and *Microcystis* cells were counted under a compound light microscope (Leica DMIL, 200× or 400× magnifications depending on cell density of the sample). All *Microcystis* cells in 15 random fields were counted and multiplied by the ratio of area of the Palmer cell to the area counted, then multiplied by 10^4 to account for the subsample volume (Wetzel and Likens, 2000).

Microcystis dry mass was determined by performing the separation procedure on sugar formalin preserved plankton tows and drawing the floating *Microcystis* portion into pre-weighed trays. Trays with *Microcystis* were dried at 65 °C for 48 h to obtain a constant mass. Linear regression analysis of cell counts and dry mass with biovolume were performed using the statistical software SAS (Statistical Analysis System, version 9.1). Tests for differences in separation efficiency due to site, month, or years in storage were performed using 3-way ANOVA in SAS.

Estimate of annual Microcystis biovolume

To obtain an estimate of annual biovolume of *Microcystis* for our sampling area in western Lake Erie that could be compared between years, we first determined *Microcystis* areal biovolume (mL/m^2) for each sampling date and site. Biovolume for each site was then plotted over the summer season, generally forming an arching curve of low biovolume in early summer, a peak, then declining biovolume in late summer. The annual *Microcystis* biovolume ($mL/m^2/y$) produced at each site was estimated by calculating the area beneath each curve using the trapezoidal rule. Finally, an annual biovolume for our sampling area was calculated by averaging across sites. Data collected from the two bay sites (MB20 and MB18) were omitted for all years because these sites were infrequently visited or plankton was not collected during the early years of this study. In most years, sampling continued into October, but in a few years (2004, 2006, 2008) the sampling season ended in mid-September. In all years, *Microcystis* blooms had disappeared by mid-October, and the latest date that *Microcystis* was observed was October 9, 2003. Therefore for the purpose of calculating annual biovolume for years in which sampling ended before October, *Microcystis* biovolume in all years was assumed to be zero on October 9.

Results

Efficiency of the flotation method, Microcystis retention by net tows, and comparisons with other methods

On average, the flotation method was able to separate 86% of the *Microcystis* colonies from the other components of plankton samples.

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Samples that had the lowest separation efficiency contained large amounts of filamentous diatoms (*Aulacoseira* sp.) and occasionally non-floating cyanobacteria (*Planktothrix* sp.), which became entangled with *Microcystis* colonies. There were no significant differences in separation efficiency due to site ($p = 0.814$), month ($p = 0.561$), or years in storage ($p = 0.207$). Although separation efficiency after adding the second separation step was not calculated, it is expected that the average final separation efficiency would be above 86%. We did not specifically investigate the mechanism that caused *Microcystis* to float. However, we noted that when *Microcystis* samples were analyzed and then re-preserved in buffered formalin that did not contain sugar, the *Microcystis* colonies lost some of their buoyancy. Sugar is added to formalin preservative specifically to prevent osmotic 'ballooning' of zooplankton (Haney and Hall, 1973), and we suspect that it may have a similar effect on preserving the integrity of gas vacuoles of cyanobacteria. The preservative may also have caused other buoyant cyanobacterial species such as *Aphanizomenon* sp. and *Anabaena* sp. to float, but these were minor components of most cyanobacterial blooms in western Lake Erie and were not captured by our plankton net to a measureable extent.

All tows collected in this study were collected during the daytime; therefore we cannot comment sufficiently on the effect of time of day or night on *Microcystis* buoyancy or separation efficiency. Informally, we noticed that the main feature that determined how quickly *Microcystis* floated to the surface was colony size. Large colonies floated almost immediately, whereas small colonies took several hours to float to the surface of the separation cone. The 2-step, 24-hour separation period that we used was judged to allow even the slowest floating colonies sufficient time to float to the top of the separation cone.

The number of cells in the concentrated *Microcystis* fraction was highly correlated with biovolume (cells = 18,611,163 (biovolume) + 165,821; $p<0.0001$; $R^2 = 0.84$; Fig. 2A), as was *Microcystis* dry weight (weight = 0.0107 (biovolume) − 0.0742; $p<0.0001$; $R^2 = 0.95$; Fig. 2B). The total biovolume of *Microcystis* colonies captured in the plankton net was highly correlated with *Microcystis* cell density (cells/m^3) in corresponding whole water column samples (density = 252,469,314 (biovolume) − 73,589,175; $p<0.0001$; $R^2 = 0.89$; Fig. 3). Therefore we conclude that total biovolume of concentrated *Microcystis* colonies from plankton tows was an acceptable surrogate for whole water cell counts and dry weight measurements.

The *Microcystis* biovolumes retained in the 112-μm mesh net did not differ significantly from biovolumes retained in the 64-μm mesh net (Mann–Whitney *U* test; $p = 0.98$). Further, it was previously determined that 112 μm mesh nets retained 93% of *Microcystis* cells in western Lake Erie compared to unstrained whole water samples (Chaffin et al., 2011). Therefore the larger mesh net was judged to be adequate for retaining most *Microcystis* colonies.

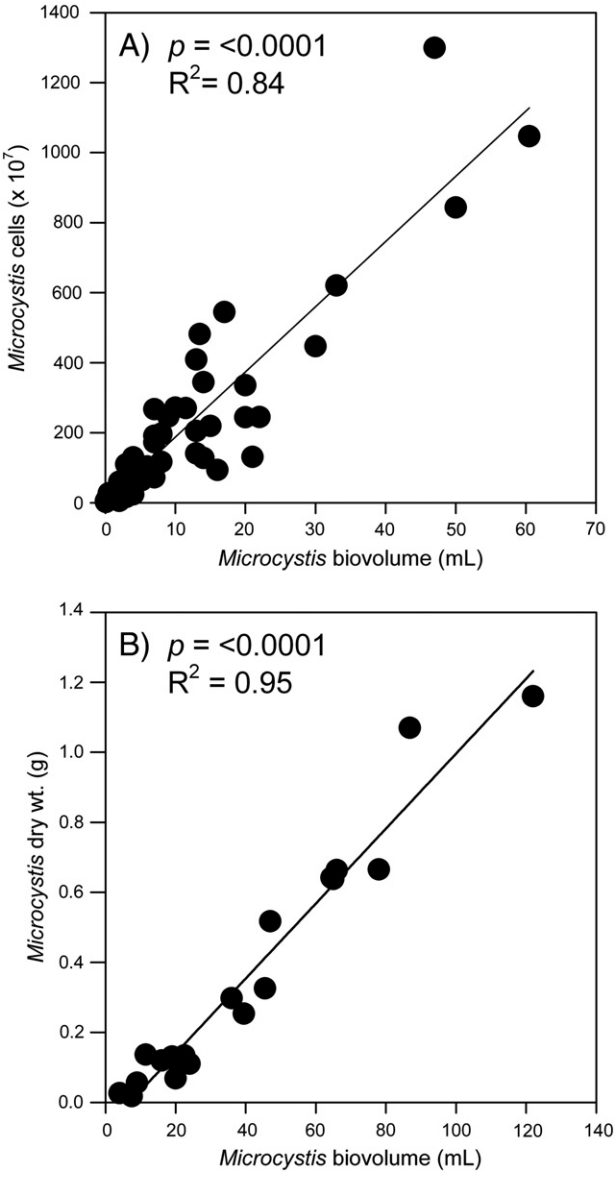

Fig. 2. Linear regressions using *Microcystis* biovolume in the separated floating fraction to predict A) *Microcystis* cell number ($n = 60$) and B) *Microcystis* dry weight ($n = 20$) in that fraction.

Seasonal, annual, and spatial estimates of Microcystis biovolume

The analysis of *Microcystis* in archived plankton tows permitted a useful retrospective analysis of seasonal patterns and inter-annual variability in western Lake Erie *Microcystis* blooms. *Microcystis* biovolume increased to a peak during the summer in all years except 2004 when biovolume was consistent from mid-July through early September (Fig. 4). The seasonal timing of peak biovolume varied, occurring in July (2005, 2007, 2010), August (2006, 2009, 2011), or September (2002, 2003, 2008). The greatest average *Microcystis* biovolume was recorded in August 2011.

To compare *Microcystis* blooms across years, biovolumes were integrated throughout the summer and averaged across sites to produce an annual estimate of the total *Microcystis* bloom. The results indicate high variability among years (Fig. 5). Relatively low bloom years (2002, 2007) are contrasted with high bloom years (2003, 2008, 2009, 2010, 2011). In particular, the 2011 bloom was 2.4 times greater than the second largest bloom (2008) and 29.0 times greater than the lowest bloom year (2002). Although the lowest biovolumes occurred in the first year of the study and the highest biovolumes were measured in the last year, there does not appear to be any predictable trend over time in *Microcystis* biovolume. However, large blooms occurred in the last 4 years of the study (2008–2011), suggesting that eutrophication may be intensifying in Lake Erie.

Microcystis biovolume also varied spatially (Fig. 6). Lowest biovolumes were generally recorded at site MB20, which is the closest to the Maumee River. Site 8M had the highest median, mean, 75th percentile, and 95th percentile *Microcystis* biovolumes when all samples were considered. Site 7M had the second highest biovolume. Sites 8M and 7M are located in a transition zone between nutrient-rich, turbid, Maumee Bay and the less productive waters characteristic of the center of the western basin (Moorhead et al., 2008). *Microcystis* may attain high density in this region because of an advantageous combination of nutrients, depth, turbidity, and mixing (Chaffin et al., 2011). *Microcystis* biovolumes generally decreased towards the center of the western basin (sites GR1 and 4P). Sites GR1 and 4P had lower medians and lower 75th and 95th percentiles compared to 8M and 7M, but had similar means because of the very high biovolumes recorded during 2011. In

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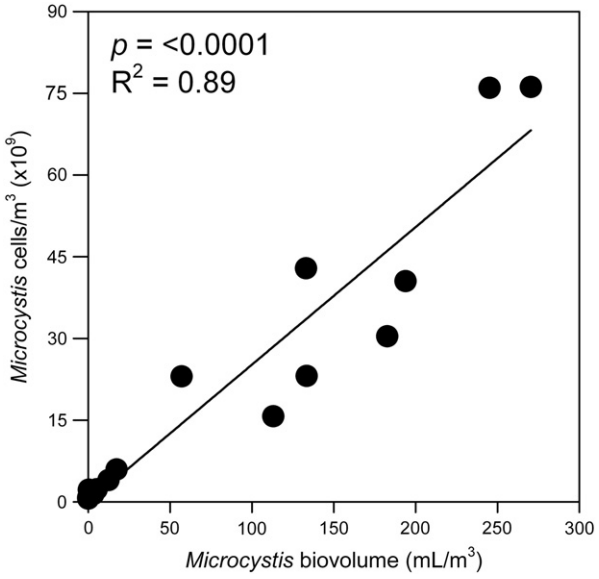

Fig. 3. Linear regression using *Microcystis* biovolume retained in vertical plankton tows to predict traditional counts of *Microcystis* cell density in corresponding whole water samples (n = 15).

the highest bloom years (2003, 2008–2011), *Microcystis* blooms were detected first in the bay-lake transition zone (e.g., sites 8M and 7M) and then a few weeks later in offshore waters and eventually covering much of the western basin. Therefore blooms in the Maumee Bay-Lake Erie transition zone may serve as an early warning for other areas of western Lake Erie.

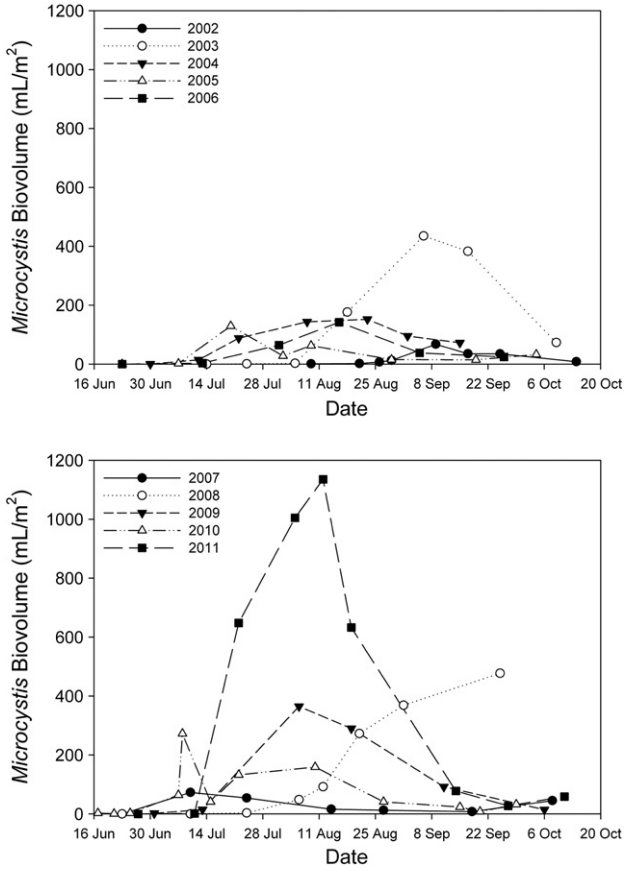

Fig. 4. Seasonal patterns of average *Microcystis* biovolume in western Lake Erie, 2002–2011. Values are the mean of four sites (8M, 7M, GR1, and 4P).

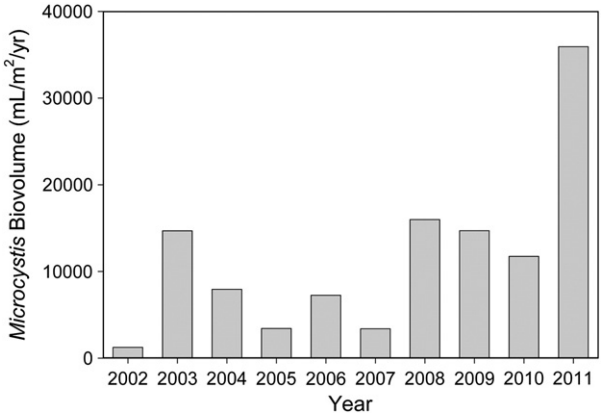

Fig. 5. Average (sites 7M, 8M, GR1, 4P) summer *Microcystis* biovolume in western Lake Erie.

Discussion

The flotation method provides a reasonably effective and convenient tool for quantifying *Microcystis* blooms in western Lake Erie and tracking blooms over space and time. There are several advantages of the method: (1) The biovolume results correlate well with conventional microscope cell counts and biomass measurements. (2) The large, vertically integrated water volume sampled by plankton nets may provide a better representation of the water column than low-volume grab samplers when *Microcystis* colonies are widely scattered, concentrated at particular depths, or surface scums are present. (3) Archived plankton tows may be used because *Microcystis* in sugar formalin remains buoyant for many years while in storage. The plankton tows collected in the early years of this study were not originally intended for the analyses of *Microcystis* but became very useful in evaluating differences in *Microcystis* biovolume among sites, seasons, and years. (4) The analysis may be performed at little expense because several samples may be processed simultaneously, largely unattended, and with minimal equipment costs.

Although we have shown this method to be effective in western Lake Erie, its applicability to other lakes has not been tested. Before applying the method elsewhere, it may be necessary to repeat some

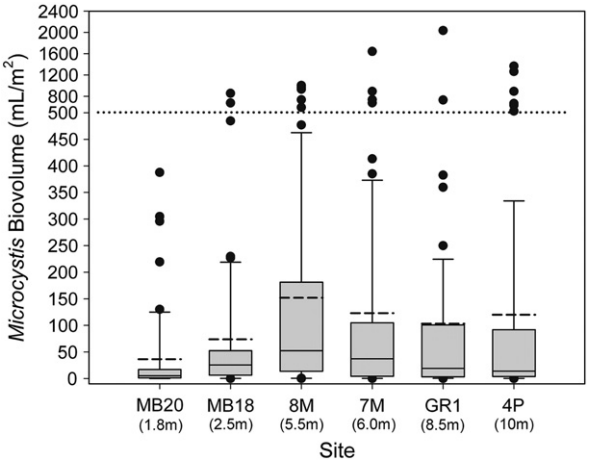

Fig. 6. Comparisons of spatial occurrence of *Microcystis* at six sites in western Lake Erie during 2002–2011. Sites are arranged in increasing distance from the mouth of the Maumee River (2 km, 7 km, 14 km, 15 km, 27 km and 31 km). Site depth is indicated below site name. The shaded boxes represent the interquartile range, and the whiskers represent the 5th and 95th percentiles. The solid and dashed lines show the median and mean, respectively. Filled circles are outliers. Note the change in scale above 500 mL/m^2.

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of the comparisons with traditional measures described above for each body of water. The size of *Microcystis* colonies is of particular concern because colony size determines how effectively colonies are captured by plankton nets. For example, in several Iowa and Missouri lakes plankton nets with 100-μm mesh size retained only about 75% of *Microcystis* as measured by microcystin toxin concentration (Graham and Jones, 2007). Although *Microcystis* tends to form large colonies in western Lake Erie (often exceeding 2 cm in length, personal observation), plankton nets may be less useful in the central basin of Lake Erie (Filbrun, unpublished data) where *Microcystis* colonies may be three times smaller than western basin colonies (Chaffin, unpublished data). Furthermore, *Microcystis* buoyancy is correlated with colony size. Large colonies of *Microcystis* tend to be several orders of magnitude more buoyant than other cyanobacteria in natural conditions (Reynolds et al., 1987) and when preserved in sugar formalin, readily separate from all other plankton in an Imhoff cone. However, we have observed that small *Microcystis* colonies float more slowly, which could increase the difficulty in separating them from other cyanobacteria with small, less-buoyant colonies, such as *Anabaena* sp. Light conditions also affect the buoyancy of cyanobacteria (Oliver and Walsby, 1984); therefore it is possible that samples collected at night might have different characteristics of separation in our procedure. Our method will be most effective in tracking cyanobacterial blooms in situations, such as western Lake Erie, where blooms are composed almost completely of large *Microcsytis* colonies. In a mixed-species cyanobacterial bloom, the method would be useful in tracking the *Microcystis* component of the bloom, but other methods would need to be employed to track the other cyanobacterial species.

Analysis of archived plankton tows using this method allowed us to determine the timing and magnitude of *Microcystis* blooms between 2002 and 2011, revealing that the bloom of 2011 was by far the most extensive of the past decade. Variation in the timing and biovolumes of blooms in western Lake Erie is likely linked to water temperature and the timing and magnitude of nutrient loading from major tributaries. For example, the 2007 bloom first peaked in early July, but then a second peak was recorded in early October. The October 2007 *Microcystis* bloom was probably a result of unusually high water temperatures for that time of year and an unusual late August storm that loaded a large amount of nutrients into the lake from the Maumee River (Richards et al., 2010). Likewise, the extremely large biovolumes recorded in the summer of 2011 followed near-record precipitation, Maumee River discharge, and nutrient loading in the late spring of that year.

For many researchers, as for us, the need for tracking blooms may become apparent only years after ecosystem changes have begun to take place. In such cases, using archived samples to extend bloom data further into the past may facilitate the exploration of the relationships between bloom formation and underlying environmental factors such as introductions of exotic species, agricultural practices, patterns of precipitation, and nutrient loading.

Acknowledgments

The authors would like to thank the numerous undergraduate and graduate assistants, technicians, and volunteers who participated in collecting samples and data over the 10-year span of this project including: Patricia Armenio, Peter Bichier, Christopher Bronish, Kristen Devanna, David Hartson, Christopher Lauber, Rachel Lohner, Joshua Morris, Abby Norton, Sarah Panek, Jill Reighard, Chad Ronyetz, Stephen Timmons, Katherine Wucusick, Christine Mayer, Stephanie Messa, and Courtney Mobilian. We thank Scott Heckathorn for the use of his laboratory in 2007. Support for this work was provided over various periods by: The Ohio Sea Grant College Program, Project R/ER-72, under grant NA16RG2252 from the National Sea Grant College Program, the Lake Erie Commission, Lake Erie Protection Fund Projects #03-19, #0-08 and #SG406-11, USEPA project #GL2009-1-2, the University of Cincinnati, the USDA Natural Resources Conservation Service (MVRC&D), and the National Science Foundation under grant no. 1039043. J. Chaffin and J. Filbrun were supported by summer fellowships through the University of Toledo's NSF-REU program (#EAR-0552552) in 2006 and 2007, respectively, and J. Chaffin was supported by an NSF GK-12 fellowship through the U. Toledo Lake Erie Center (grant #DGE-0742395) in 2010–2011. This is contribution 2012–15 of the Lake Erie Center.

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